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EXAMINER	
WOOLWINE, SAMUEL C	
ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/520,693	DIXON ET AL.	
	Examiner	Art Unit	
	SAMUEL WOOLWINE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 December 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status

As per the agreement made during the interview on 12 November 2008 (see *Interview Summary* mailed 11/18/2008), Applicant's response received 12/17/2008 has been entered for consideration.

The rejections of record are maintained as reiterated below. Applicant's arguments and declaration filed 12/17/2008 will be addressed following the rejections (the arguments filed 07/07/2008 were already addressed in the Office action mailed 09/18/2008). As no new grounds of rejection have been made, this action is made FINAL.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-6 and 8-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Richardson et al (WO 01/06004 A2, cited on the IDS of 8/28/2006) in view of Gu et al (US 2003/0180737).

With regard to claim 1, Richardson teaches a method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample (e.g. page 25, lines 27-29), the method comprising the steps of:

(i) reverse transcribing the mRNA species using a heeled 5'-amplification primer (FAP-RAND) (e.g. "first heeled primer population"; page 25, lines 30-31)

and a heeled 3'-amplification primer (TAP-RT) (e.g. "second heeled primer population"; page 25, lines 32-33),

wherein each primer sequence is unique (it is noted in paragraph [0007] of Applicant's published application: "[a]nother advantage is that the production of complex products is minimised, due in part to the use of unique sequences in FAP and TAP which are absent from the genome being investigated"; however, Richardson clearly teaches this concept in, for instance, Example V, pages 55-58, wherein the primer sequences were chosen based on their absence from the genome being investigated (see page 55, lines 29-30 and page 56, lines 25-26; also note that Richardson teaches that the heel sequence of the first heeled primer "is not complementary to the first strand cDNA nor the mRNA molecules initially present in the sample" (page 39, lines 13-15) and that the heel sequence of the second heeled primer "is not complementary to the mRNA molecules present in the sample or with the first strand cDNA molecules synthesized at step a)", thus the heel sequences are "unique"),

and either or each heel sequence includes a RNA polymerase promoter site (see for example page 39, lines 16-17 (for the "first heeled primer") and page 40, lines 29-31 (for the "second heeled

primer"); note also Richardson's Example V uses a T7 RNA polymerase promoter sequence for the first primer (page 55, lines 24-33) and a T3 RNA polymerase promoter sequence for the second primer (page 56, lines 24-31)),

and the FAP includes a variable sequence (Richardson teaches each primer as having a variable sequence; see page 39, lines 19-22 (for the first heeled primer) and page 40, lines 20-22 (for the second heeled primer)),

whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence (page 25, lines 32-33 and page 26, line 21 through page 28, line 2; page 39, lines 19-22; page 40, lines 20-22; it is clear that the products synthesized are determined in part by the variable sequences present in the heeled primers);

and (ii) of amplifying the cDNA using primers sufficiently complementary to the primer sequences FAP and TAP, within FAP-RAND and TAP-RT (26, lines 4-7; page 31, line 26 through page 32, line 2).

With regard to claim 2, Richardson teaches in vitro transcription (see page 37, lines 12-25 and page 38, lines 5-24, for example).

With regard to claim 3, Richardson's Example V uses a T7 RNA polymerase promoter sequence for the first primer (page 55, lines 24-33) and a T3 RNA polymerase promoter sequence for the second primer (page 56, lines 24-31).

With regard to claim 4, see page 38, lines 5-24. Richardson teaches incorporation of an RNA polymerase promoter in the [first heeled] primer allows synthesis of complementary RNA, whereas incorporation of an RNA polymerase promoter in the second heeled primer allows synthesis of sense RNA. Either of these embodiments generates a strand-specific library.

With regard to claim 5, Richardson teaches using the method for the production of a subtracted library (page 44, lines 6-17; the "two cell populations" is implied by the "two different samples" at line 13).

With regard to claim 6, Richardson teaches immobilizing the polynucleotide products of his method to an array (page 37, lines 23-25). Note that there is no explicit definition of "cloning" in Applicant's disclosure. Therefore, the generation of multiple copies of a particular polynucleotide, as in the method of Richardson, can be considered "cloning".

With regard to claim 8, Richardson teaches using a sample from patch clamp harvesting (page 59, lines 14-28).

With regard to claims 9 and 10, Richardson teaches the first and second heeled primers having cleavage sites at the 3' ends of the heel sequences (page 39, lines 28-31 and page 40, lines 23-26).

With regard to claim 11, Richardson teaches the cleavage sites in the first and second heeled primers can be identical (page 42, lines 25-27).

With regard to claim 12, Richardson teaches the cleavage sites in the first and second heeled primers can be different (page 42, lines 28-30).

With regard to claim 13, Richardson teaches an embodiment which includes an additional step of treating the polynucleotides with an agent (restriction enzyme) that cleaves at the cleavage site (page 35, lines 29-34).

With regard to claim 14, Richardson teaches up to 50 amplification cycles (page 31, lines 13-15).

With regard to claim 15, Richardson teaches each amplification cycle comprises the steps of obtaining single-stranded DNA molecules at a temperature between 80°C and 95°C (which overlaps

the claimed range; page 32, lines 5-6), annealing the single-stranded DNA molecules at a temperature between 65°C and 75°C (which overlaps the claimed range; page 32, lines 10-12), and elongating the annealed DNA molecules at a temperature between 65°C and 75°C (which encompasses the claimed range; page 32, lines 10-12).

With regard to claim 16, Richardson teaches each of these characteristics of the first heeled primer on page 39, lines 10-22:

> In another aspect of the third embodiment of the present invention, the first heeled primer population consists of a population of nucleic acids comprising, from 5' end to 3' end:
>
> (i) a heel sequence of 15 to 22 nucleotides in length which is not complementary to the first strand cDNA nor the mRNA molecules initially present in the sample;
>
> (ii) An option but preferably present RNA polymerase promoter site;
>
> (iii) an oligo dT sequence of 15 to 35 nucleotides in length; and
>
> (iv) a variable sequence of 2-4 nucleotides in length that can hybridize to a mRNA molecule at the 5'end of the poly-A tail thereof,
>
> wherein substantially every possible variable sequence combination is found in said first heeled primer population.

With regard to claims 17 and 18, Richardson teaches detection of the sequence of interest according to the recited methods (page 5, lines 11-23):

Increase the number of nucleotide sequences corresponding to the mRNA species present in a sample is intended to designate an increase in nucleotide sequence to obtain a number of copies which is sufficient to allow at least one of the following methods:

(i) detection of the sequence of interest with specific oligonucleotide probes;

(ii) amplification of the sequence of interest with specific oligonucleotide primers;

(iii) cloning of the DNA molecules obtained in a replication and/or expression vector, or

(iv) In vitro RNA transcription, either for hybridization assays of for further reverse transcription optionally using unlabelled or labeled substrates followed by gene specific PCR or hybridization.

With regard to claim 19, Richardson teaches an example in which the first heeled primer comprises a T7 promoter (page 55, lines 26-33) and the second heeled primer comprises a T3 promoter (page 56, lines 25-29).

With regard to claim 20, Richardson teaches production of RNA "run-offs" using T7 and T3 RNA polymerases (see pages 53-54 and especially page 57, lines 17-32).

Richardson does not teach that the reverse transcribing with the first and second heeled primers to produce double-stranded cDNA was performed in a single step, as recited in amended claim 1. Rather, Richardson teaches adding the second primer after the first strand cDNA was synthesized (e.g. page 26, line 31).

Gu teaches synthesizing first and second strand cDNA in a single step (e.g. paragraph [0010]; paragraphs [0167]- [0168]) using thermostable DNA polymerases with reverse transcriptase activity.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to carry out the first and second strand cDNA synthesis using the "single-pot" method of Gu when practicing the method of Richardson, since this would have been more efficient. Also, Gu teaches this method can be carried out at elevated temperatures, thus overcoming the problem of RNA secondary structure during reverse transcription (see paragraph [0004]).

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Richardson et al (WO 01/06004 A2, cited on the IDS of 8/28/2006) in view of Gu et al (US 2003/0180737) as applied to claims 1-6 and 8-19 above and further in view of Fend et al (American Journal of Pathology, vol 154, no 1, pp 61-66, 1999, prior art of record).

The teachings of Richardson and Gu have been discussed. These references do not teach using a sample in his method that was obtained by laser capture microdissection.

Fend teaches a method for laser capture microdissection to obtain single cells for mRNA analysis (see entire article). Fend teaches that analysis of gene expression in normal or pathologically altered cells can "lead to the establishment of genetic fingerprints of neoplasms" (page 61, 1st paragraph following abstract). Fend also teaches that microdissected samples allows the isolation of morphologically identified cell populations down to the single-cell level, which overcomes the problem of tissue heterogeneity that can confound the attempt to differentiate gene expression patterns between neoplastic and non-neoplatic tissue (page 61, 1st and 2nd paragraphs following abstract).

It would have been prima facie obvious to use Richardson's method (modified according to the teachings of Gu) to analyze samples obtained by laser capture microdissection, since Fend clearly

teaches the value of laser capture microdissected samples and since Richardson was clearly interested in analyzing the RNA content of single cells (e.g. see page 3, lines 26-29, page 43, lines 32-35, page 44, lines 34-35, page 45, lines 1-3, page 58, lines 11-16). Thus it would have been obvious to one of skill in the art at the time the invention of the instant application that Richardson's method was ideally suited for the analysis of laser capture microdissection samples.

Response to Arguments

Applicant's arguments and declaration filed 12/17/2008 have been fully considered but they are not persuasive.

Applicant argues on page 6:

> Applicants respectfully maintain that for their claimed invention, in order to establish a *prima facie* case of obviousness, it is necessary for the Patent Office to show that the ordinarily skilled artisan would have had some suggestion or motivation to combine the cited references.

The rejection pointed out a motivation: performing the 1st and 2nd strand cDNA synthesis in the manner taught by Gu would have been more efficient than performing separate steps.

Applicant argues at page 7:

> Applicants respectfully maintain that the ordinarily skilled artisan would not have been able to predict the outcome of combining multiple chemical reaction steps into a single step. The ordinarily skilled artisan would not have looked to combine the teachings of the Richardson *et al.* and Gu *et al.* publications as there was no teaching or suggestion in the art that such a combination would yield desirable results, or that methods resulting from the combination would even work at all.

The examiner respectfully disagrees. Gu expressly teaches that reverse transcription and PCR (which would inherently form the first and second strands of cDNA) could be performed as a

single pot reaction. Paragraph [0010]: "In some embodiments, the reverse transcription and amplification reactions are conducted in the same reaction buffer (i.e., a single pot reaction is performed)." Paragraph [0165], emphasis provided: "The present invention contemplates single-reaction RT-PCR wherein reverse transcription and amplification are performed in a single, continuous procedure."

Furthermore, Gu describes a working example (Example 14) where such is performed (page 25, Example 14: "nTvu Reverse Transcriptase Functions in a Single-Step RT-PCR Reaction"). Thus, Gu *evidences* that it was possible to combine "multiple chemical reaction steps into a single step", thereby providing a reasonable expectation of success. There is no rational explanation provided by Applicant, nor any scientific reason of which the examiner is aware, why the combination of Richardson and Gu would not be expected to work merely because Richardson recites particular structural features on the primers (i.e. "heels", "unique sequences"). Richardson successfully used the primers in *separate* RT and PCR steps, and Gu teaches that RT and PCR steps could be combined. Hence the successful outcome of the combination was predictable.

Applicant argues on page 7:

> Also under these rejections, the Examiner asserts that no data has been provided to establish that the claimed method is more efficient than the methods in the cited references. However, under the authority of *In re Marzocchi*, 169 USPQ 367 (CCPA 1971), Applicants' statements concerning the claimed invention must be taken as true unless the Patent Office can recite specific reasons to doubt the validity of those statements. The Examiner has not provided evidence or teachings to doubt the validity of Applicants' statements concerning the invention.

The issue in Marzocchi was whether the claims were enabled.

It is submitted that the instant situation differs from Marzocchi, in that Applicant is relying on an asserted (albeit in the specification as filed, as well as in arguments subsequently presented) "unexpected result" as evidence of nonobviousness. Applicant argues on page 8:

> As indicated in the Declaration under 37 CFR §1.132 by Dr. Richardson submitted with this Response, Applicants' claimed invention is surprisingly simpler and more efficient (*e.g.*, reverse transcription proceeds more efficiently), and requires fewer amplification cycles, than methods of the Richardson *et al.* publication. There is nothing in the art that would lead an ordinarily skilled artisan to predict that Applicants' claimed invention would be more efficient or require fewer amplification cycles.

It is respectfully submitted that combining two steps into one (conducting first and second strand cDNA synthesis "in a single step") would have been *expected* to be simpler and more efficient (from a labor-saving perspective). As to Applicant's assertion that fewer amplification cycles were needed, MPEP 716.01(c)(I) clearly states (emphasis provided):

"Objective evidence which must be factually supported by an appropriate affidavit or declaration to be of probative value includes evidence of unexpected results... See, for example, *In re De Blauwe*, 736 F.2d 699, 705, 222 USPQ 191, 196 (Fed. Cir. 1984)". In *De Blauwe* the Apellants argued that any case of *prima facie* obviousness had been rebutted because the claimed invention unexpectedly overcame a problem, to which the Solicitor countered there was no *objective evidence* in the record that the result was in fact a due to the claimed invention or that the result was unexpected. The Appellants, like Applicant, argued that their assertions of unexpected results were supported by objective evidence, relying on passages from the specification. The court sided with the Solicitor on this matter stating: "It is well settled that unexpected results must be established by factual evidence. Mere argument or conclusory statements in the specification does not suffice."

Furthermore, MPEP 716.02(b) holds:

"The evidence relied upon should establish "that the differences in results are in fact unexpected and unobvious and of both statistical and practical significance." *Ex parte Gelles*, 22 USPQ2d 1318, 1319 (Bd. Pat. App. & Inter. 1992)"

Also, MPEP 716.02(d) provides:

"Whether the unexpected results are the result of unexpectedly improved results or a property not taught by the prior art, the "objective evidence of nonobviousness must be commensurate in scope with the claims which the evidence is offered to support. ""

Here, there is no comparative data that conducting first and second strand cDNA synthesis "in a single step" (which was the only identifiable distinction between claim 1 and Richardson) is what resulted in the asserted unexpected result (i.e. a need for fewer amplification cycles). It is not even clear from Applicant's disclosure whether this was the factor leading to the asserted unexpected result (and hence, whether the claims are commensurate in scope with the unexpected result). Furthermore, it cannot be determined whether the result is of "statistical and practical significance".

Applicant also argues on page 8:

> as Dr. Richardson indicates in the attached Declaration, by comparison with the procedure in the Richardson *et al.* publication, the present invention advantageously avoids the need for rare restriction sites, and it allows for the inclusion of specific restriction sites for lambda cloning.

This argument is also presented in item 4 of the declaration.

Note however from page 16 of Richardson ("Second Embodiment"):

b) synthesizing second cDNA strands from said first strand cDNA sequences using a second heeled primer population, wherein each of the primers of said first, and/or second heeled primer population optionally contains a rare cleavage site in particular a rare restriction site located at the 3' end of its heel sequence;

Hence, Richardson stated that the "rare cleavage site" was optional (not "needed"). In fact, the "First Embodiment" of Richardson does not even mention "rare cleavage site". Furthermore, the instant claims are silent in regards to a "rare cleavage site". Hence this issue does not distinguish the instant claims from Richardson.

Applicant also argues on page 9:

Another advantage of the claimed method as explained by Dr. Richardson in his Declaration is that the production of complex products is minimized, due in part to the use of unique sequences in the heeled 5'-amplification primer (FAP) and the heeled 3'-amplification primer (TAP) which are absent from the genome being investigated. Moreover, while the procedure described in the Richardson *et al.* publication uses a single primer to amplify the products after reverse transcriptase and second strand synthesis, Dr. Richardson explains that the present invention provides the significant advantage that two separate primers of unique sequence are used (see point 5 of Dr. Richardson's Declaration).

This argument is also presented in item 5 of the declaration.

Note however from page 9 of Richardson:

However, the heel regions may simply comprise sequences absent from the genome of the organism from which the sample is taken.

And page 11:

Step c):

The amplification reaction of step c) is performed with a pair of oligonucleotide primers that respectively comprise at least a portion of the heel sequence of the first and second heeled primers that are defined above.

The first primer of step c) is preferably the heel of the first heeled primer. The second primer of step c) is preferably the heel of the second heeled primer.

Hence Richardson teaches the heel sequences are "not present in the genome", and this issue does not distinguish the instant claims over Richardson. Furthermore, the term "pair of primers" used in Richardson certainly renders obvious the case where the two primers are different. Moreover, the claims at issue do not clearly recite that the two primers *are* in fact different. Hence, these issues do not distinguish the instant claims over Richardson.

With regard to the points enumerated in the declaration, items 4 and 5 have already been treated. With regard to item 3, it would not have been unexpected that synthesizing the 1st and 2nd strands of cDNA in a single step (the only difference between the methods *as claimed* and the disclosure of Richardson) would be simpler and more efficient. The issue of requiring fewer amplification cycles as a secondary consideration has been discussed above.

With regard to item 6, it is asserted that synthesizing the 1st and 2nd strands of cDNA in a single step has nothing to do with the ability to add restriction sites to the primers, cloning, manufacturing single cell libraries or carrying out other procedures such as laser capture microdissection. If the assertion is that the cloning, single cell libraries and the ability to use laser capture microdissection samples (e.g. which contain minimal amounts of starting material) are made possible because the claimed methods yield higher amounts of product for a given number of cycles

of amplification, this asserted "unexpected result" (i.e. fewer amplification cycles needed) and the lack of objective evidence has been discussed.

Conclusion

All claims are drawn to the same invention claimed in the application prior to the entry of the submission under 37 CFR 1.114 and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the application prior to entry under 37 CFR 1.114. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action after the filing of a request for continued examination and the submission under 37 CFR 1.114. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/
Examiner, Art Unit 1637

/Young J Kim/
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